UNIVERSITI PUTRA MALAYSIA

PRODUCTION, PROPERTIES AND APPLICATIONS OF MYCELIUM-BOUND LIPASE OF A LOCALLY ISOLATED STRAIN OF *ASPERGILLUS FLAVUS* LINK

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By

KAMARIAH LONG

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" AL HAMDULILLAH "

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

µg	microgram
mg	milligram
g	gram
mm	millimetre
cm	centimetre
mL	millilitre
L	litre
sec	second
min	minute
h	hour
µmol	micromole
mM	millimolar
M	molar
v/v	volume/volume
w/v	weight/volume
rev/min	revolution per minute
MW	molecular weight
sp	species
C_6	caproic acid
C_8	caprylic acid
C_{10}	capric acid
C_{12}	lauric acid
C_{14}	myristic acid
C_{18}	palmitic acid
$C_{18:1}$	oleic acid
$C_{18:2}$	linoleic acid
$C_{18:3}$	linolenic acid
OOO	trioleoyl glycerol
OOC	dioleoyl-capryl glycerol
OCC	dicapryl-oleoyl glycerol

CCC	tricapryl glycerol
OOL	dioleoyl-lauryl glycerol
OLL	dilauryl-oleoyl glycerol
LLL	trilauryl glycerol
LLM	dilauryl-myristroyl glycerol
LMM	dimyristroyl-lauryl glycerol
MMM	trimyristroyl glycerol
CCL	dicapryl-lauryl glycerol
CCLn	dicapryl-linoleoyl glycerol
CLnLn	dilinoleoyl-capryl glycerol
LnLnLn	trilinoleyl glycerol
OOP	dioleoyl palmitoyl glycerol
OPP	dipalmitoyl-oleoyl glycerol
PPP	tripalmitoyl glycerol
LLP	dilauryl-palmitoyl glycerol
LPP	dipalmitoyl-lauryl glycerol
OOLn	dioleoyl-linoleoyl glycerol
OLnLn	dilinoleoyl-oleoyl glycerol
HPLC	high performance liquid chromatography
GC	gas ghromatography
DSC	differential scanning calorimetry
°C	degree celcius
EDTA	ethylenediamin tetraacetic acid
PMSF	phenylmethylsulfonyl fluoride
FAME	fatty acid methyl ester
PUFA	polyunsaturated fatty acid
%	percent
UFA	unsaturated fatty acid
SFA	saturated fatty acid
TG	triglyceride
DG	diglyceride
MG	monoglyceride
FA	fatty acid

Abstract of the Dissertation presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosophy.

PRODUCTION, PROPERTIES AND APPLICATIONS OF MYCELIUM-BOUND LIPASE OF A LOCALLY ISOLATED STRAIN OF *ASPERGILLUS FLAVUS* LINK

By

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One of the most promising processes using lipase which will offer great potential application especially in term of cost reduction, is the use of mycelium-bound lipase (naturally immobilized lipase). However, there has been little work reported on the technology using mycelium-bound lipase (naturally immobilize) and work using it, is limited to a few microorganisms. This study was conducted with the aims to identifying a new source of mycelium-bound lipase, and consequently to study it's production, properties and applications.

Aspergillus flavus, isolated from copra meal, produces a lipase (EC 3.1.1.3) which is partly bound to the mycelium. The production of the mycelium-bound lipase is concomitant with growth, and declines when growth ceases. Maximum productivity of the enzyme is obtained when the culture is incubated at 30°C, an initial culture pH of 6.5 and with 2% (w/v) each of corn oil and yeast extract as carbon and organic nitrogen sources, respectively. Yeast extract affects not only the production of lipase

but also the secretion of proteases into the culture medium. The presence of EDTA improved the productivity of the mycelium-bound lipase by 26% although growth of *A. flavus* was inhibited by 11%. The addition of Tween 80, into culture medium, decreased the activity of mycelium-bound lipase.

The lipase that is bound to the mycelia has various degrees of binding. Twenty-eight percent of its' activity was easily released after washing n-hexane deffated mycelia with water. The rest was quite easily released by treating the mycelia with 0.05 M Tris-HCl buffer pH 8.2, at 35°C for 90 min, 200 rev/min. However, 7% is tightly bound and released only upon treatment with a lytic enzyme preparation from *Trichoderma harzianum* (Sigma L-2265). Non-polar solvents are better defatting agents. The mycelium-bound lipase of *A. flavus* is stabilised by cross-linking using glutaraldehyde, methylglyoxal or ethylenediamine. The treatment caused inactivation of the enzyme except for mycelia treated with methylglyoxal whose activity was enhanced by up to 48%. On the other hand, lipase from glutaraldehyde-treated mycelia is physically more stable than the lipase from methylglyoxal-treated mycelia. The stability of untreated and glutaraldehyde-treated mycelia-bound lipase in a semi-continuous packed-bed reactor was examined and it was found that the hydrolytic and synthesis activities of lipases decreased and after 6 cycles of use the activity remained unchanged (20 cycles)

The bound lipase, when released into solution, was unstable and lost almost all of its activity after 14 days storage at 4°C. The loss of activity of extracted lipase is due to inactivation by co-extracted proteases. The extracted enzyme has temperature and pH optima at 30°C and 8.0, respectively. The lipase could be separated from the

metallo-proteases by acetone fractionation. The apparent K_m value of extracted lipase (3.92 mg/mL) towards coconut oil was three times lower than that of the mycelium-bound lipase (11.76 mg/mL).

A. flavus mycelium-bound lipase demonstrates a high preference for hydrolytic activity towards low molecular weight TG and discriminates triunsaturated TG i.e. OOO. A greater discriminating degree of its lipase towards triolein was shown when the enzyme was reacted with low molecular weight triglycerides, and was less shown when reacted with PPP. Similar observations were noted when the mycelium-bound lipase was used to catalyse a reaction containing coconut oil with palmitic acid or oleic in n-hexane. The lipase displays a strong specificity towards the outer fatty acid of triglycerides (1,3 specific) and loses activity for tributryin once it has been extracted. It hydrolyses coconut oil faster than palm olein, followed by corn oil, rapeseed oil, soybean oil and, cottonseed oil.

The ability of the mycelium-bound lipase to modify vegetable oils by incorporation of exogenous fatty acid into the triglycerides in medium containing n-hexane was studied. Reaction between cottonseed oil and lauric acid gave the highest percent of incorporation (18%) of the acid followed by soybean oil with lauric acid (16%) and coconut oil with oleic acid (16%). The degree of incorporation of lauric acid into palm olein and quality of the oil could be improved with the addition of molecular sieve at specific times of reactions. In all cases, there were increases in the concentrations of several existing triglycerides and the formation of new triglycerides. DSC analysis shows that the heating thermogram of acidolysed vegetable oils exhibit broad melting ranges.